an effective amount of IL-3 wherein said effective amount is in the range of 10 ng/mL to about 100 ng/mL.

42. The method according to claim 37, wherein the effective amount of IL-6 is in the range of about 10 ng/mL to about 100 ng/mL

43. The method according to claim 37, wherein the TPO is provided as a mimetic.

44. The method according to claim 37, wherein said human hematopoietic cells are $CD34^{+}Thy\text{-}1^{+}$ Lin^{-} cells.

45. The method according to claim 37, further comprising culturing the hematopoietic cells in the presence of fibronectin or RetroNectin™.

46. The method according to claim 37, wherein the heterologous gene is a marker gene.

47. The method according to claim 37, wherein the heterologous gene is a therapeutic gene.

RESPONSE

Applicants affirm the provisional election, made with traverse, to prosecute the invention of Group III, claims 18 – 30 directed to a method of modifying hematopoietic stem cells.

Claims 18 – 20, 23 – 28, and 31 – 47 are pending in this application. Applicants have canceled claims 1 – 17 as directed to the non-elected invention. Additionally, claims 21, 22, 29, and 30 have been canceled without prejudice. Applicants reserve the right to file further applications on any subject matter disclosed in the specification but not specifically claimed in the pending application.

Applicants have amended claims 18 – 20 and 23 – 27. Claims 31 – 47 have been added by this amendment. A clean copy of the pending claims has been attached hereto for the Examiner's

convenience.

Independent claims 18 and 23 have been amended to recite that the hematopoietic stem cells are human cells. The gene delivery vehicle has been further defined as a vector selected from the group consisting of retroviral vectors, adenoviral vectors, and adeno-associated viral vectors. Support for these vectors can be found at page 21, starting at line 17 through page 23, and further at pages 12 and 13. Effective concentration ranges have been provided for the cytokines, and support for the claimed ranges is found at page 11, lines 18 – 27. The polynucleotide sequence has been defined as encoding a heterologous gene, and support is found at page 23, lines 11 – 15. Additionally, a second step has been added directed to obtaining genetically modified human hematopoietic stem cells.

Dependent claims 19, 20, 24, 25, 26, 27, and new claim 31 include a recitation of the concentration range of the cytokines. Support for the ranges can be found at page 11, line 18 – 28, page 19, lines 7 – 10 and in the examples.

New claims 32 - 36 are dependent on claim 23. Claim 32 further limits the vector to a retroviral vector. Claim 33 is directed to the heterologous gene as a marker. Claim 34 is directed to further expanding the genetically modified hematopoietic cells. Support can be found at page 9, lines 10 – 14. Claim 35 recites that the hematopoietic cells are $CD34^{+}Thy\text{-}1^{+}Lin^{-}$ cells, and support can be found in the original claims and in the specification at page 4, lines 29 – 30 and page 15. Claim 36 recites that the hematopoietic cells are further cultured in the presence of fibronectin. Support can be found in the original claims and in example 9.

New claims 37 – 47 further define the invention and are directed to a method of transducing mammalian $CD34^{+}$ hematopoietic cells cultured with TPO, FL, and IL6 with a retroviral vector. In general, support for these claims can be found in the specification in the original claims; page 11, lines 18 – 28; pages 20 and 21; and at page 23 starting at line 13 through page 24. The use of a mimetic for TPO is supported by example 6, page 40 of the specification.

The Examiner has rejected claim 21 under 35 U.S.C. 112, 2nd paragraph and claims 18 – 30 under 35 U.S.C. 112, 1st paragraph. There are no cited prior art rejections.

Applicants have canceled claims 21 and 29. These claims contained a typographical error - "form". It is submitted the rejection under 35 U.S.C. section 112, 2nd paragraph is moot in light of the cancellation of claims 21 and 29.

With respect to the rejection under 35 U.S.C. section 112, 1st paragraph, the Examiner states, the specification as filed provides enablement for a method of culturing $CD34^{+}$ $Thy\text{-}1^{+}Lin^{-}$ cells from adult human bone marrow in specific combinations of IL-3, IL-6, LIF, TPO, FL and KL which show evidence of success, i.e. as claimed to provide "an effective amount" and for expression of the Lyt2 construct exemplified.

Applicants agree that the specification provides enablement for the above, however Applicants contend enablement is provide for a broader invention. In the first instance, Applicants should not be limited to claims reciting $CD34^{+}Thy\text{-}1^{+}Lin^{-}$ cells. While this may be a preferred expression profile of human stem cells, it is not necessarily the only expression profile of human HSCs. As stated at page 4, line 27 – 29 of the specification, in one embodiment the hematopoietic stem cells are human hematopoietic stem cells, preferably $CD34^{+}$, more preferably $CD34^{+}Thy\text{-}1^{+}$ and even more preferably $CD34^{+}Thy\text{-}1^{+}Lin^{-}$. Applicants have included the recitation of "human" with respect to the hematopoietic stem cells.

Additionally, Applicants contend that the source of human hematopoietic stem cells should not be limited to adult human bone marrow. It is well known to those of ordinary skill in the art that sources of HSCs include bone marrow, both adult and fetal, mobilized peripheral blood, umbilical cord blood, fetal liver and other sources. Reference is made to page 15 of the specification wherein various sources of hematopoietic stem cells are disclosed. The specific examples in the instant specification illustrate results obtained from both bone marrow experiments, e.g. examples 1 – 5, and mobilized peripheral blood experiments, e.g. examples 6 – 9.

Applicants have added concentration ranges for the various claimed combinations of cytokines. While Applicants contend that a gene delivery vehicle including a DNA vector and a liposome delivery vehicle may be used to transfer a heterologous gene to the target cell, to further expedite prosecution of this application, Applicants have limited the instant claims, without prejudice, to a vector selected from retroviral vectors, adeno-viral vectors and adeno-associated viral vectors. The vector includes a polynucleotide sequence encoding a heterologous gene wherein the heterologous gene may be a marker gene, a therapeutic gene or both. Applicants contend the claims should clearly not be limited to expression of Lyt2. One of ordinary skill in the art could readily determine the level of expression of a heterologous gene from human hematopoietic cells cultured according to the invention by following the teachings of the specification and using well known techniques for measurement of gene expression.

Applicants respectfully request the withdrawal of all rejections under 35 U.S.C. section 112, 1st paragraph in view of the claim amendments and arguments presented herein above. Allowance of claims 18 – 20, 23 – 28 and 31 - 37 is kindly solicited.

Respectfully submitted,

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CLEAN COPY OF PENDING CLAIMS (18 – 20, 23 – 27 and 31 - 47:

18. A method for genetically modifying human hematopoietic stem cells, comprising
(a) contacting a vector comprising a polynucleotide sequence encoding a heterologous gene with a population of hematopoietic stem cells cultured in the presence of an effective amount of a mpl ligand and a flt3 ligand each provided in a concentration range of about 0.1 ng/mL to about 500 ng/mL,
wherein said vector is selected from the group consisting of retroviral vectors, adenoviral vectors, and adeno-associated viral vectors; and
(b) obtaining modified human hematopoietic stem cells.

19. The method according to claim 18, further comprising culturing the hematopoietic stem cells in the presence of a c-kit ligand in a concentration range of about 5 ng/mL to about 200 ng/mL.

20. The method according to claim 19, further comprising culturing the hematopoietic stem cells in the presence of a interleukin 3 (IL3) in a concentration range of about 5 ng/mL to about 200 ng/mL.

23. A method for genetically modifying human hematopoietic stem cells, comprising
(a) contacting a vector comprising a polynucleotide sequence encoding a heterologous gene with a population of hematopoietic stem cells cultured in the presence of an effective amount of a thrombopoietin ligand (TPO), a flt3 ligand (FL), and interleukin 6 (IL6) each provided in a concentration range of about 0.1 ng/mL to about 500 ng/mL, wherein said vector is selected from the group consisting of retroviral vectors, adenoviral vectors, and adeno-associated viral vectors; and
(b) obtaining modified human hematopoietic stem cells.

24. The method of claim 23, further comprising culturing the stem cells in the presence of an effective amount of leukemia inhibitory factor (LIF) wherein said effective amount is in the range of 5 ng/mL to about 200 ng/mL.

25. The method of claim 23, further comprising culturing the stem cells in the presence of an effective amount of interleukin 3 (IL3) wherein the effective amount is in the range of about 10 ng/mL to about 100 ng/mL.

26. The method of claim 23, further comprising culturing the stem cells in the presence of a c-kit ligand wherein said effective amount is in the range of 5 ng/mL to about 200 ng/mL.

27. The method of claim 25, further comprising culturing the stem cells in the presence of a c-kit ligand wherein said effective amount is in the range of 5 ng/mL to about 200 ng/mL.

31. The method according to claim 23, wherein the effective amount of TPO and FL individually is in the range of about 5 ng/mL to about 200 ng/mL and the effective amount of IL6 is in the range of about 10 ng/mL to about 100 ng/mL.

32. The method according to claim 23, wherein the vector is a retroviral vector.

33. The method according to claim 23, wherein the heterologous gene is a marker gene.

34. The method according to claim 23, further comprising expanding the modified human hematopoietic cells.

35. The method according to claim 23, wherein the human hematopoietic cell is a $CD34^+$ $Thy\text{-}1^+$ Lin^- cell.

36. The method according to claim 23, further comprising culturing the hematopoietic stem cells in the presence of fibronectin or Retronectin™.

37. A method of transducing mammalian $CD34^+$ hematopoietic cells including a subpopulation of hematopoietic stem cells comprising,

(a) obtaining a source of hematopoietic cells including the subpopulation of hematopoietic stem cells;

(b) culturing said cells with the cytokines TPO, FL and IL-6, individually provided in the range of about 0.1 ng/mL to about 500 ng/mL;

(c) infecting the cultured cells with a retroviral vector including a polynucleotide sequence encoding a heterologous gene; and

(d) obtaining transduced cells wherein said gene is expressed.

38. The method according to claim 37, wherein the TPO, FL and IL-6 are individually provided in the range of about 5 ng/mL to about 200 ng/mL.

39. The method according to claim 37, further comprising culturing the cells in the presence of an effective amount of leukemia inhibitory factor (LIF) wherein said effective amount is in the range of 5 ng/mL to about 200 ng/mL.

40. The method according to claim 37, further comprising culturing the cells in the presence of an effective amount of IL-3 wherein said effective amount is in the range of 10 ng/mL to about 100 ng/mL.

41. The method according to claim 39, further comprising culturing the cells in the presence of an effective amount of IL-3 wherein said effective amount is in the range of 10 ng/mL to about 100 ng/mL.

42. The method according to claim 37, wherein the effective amount of IL-6 is in the range of about 10 ng/mL to about 100 ng/mL

43. The method according to claim 37, wherein the TPO is provided as a mimetic.

44. The method according to claim 37, wherein said human hematopoietic cells are $CD34^{+}Thy\text{-}1^{+}$ Lin^{-} cells.

45. The method according to claim 37, wherein the human hematopoietic cells are further cultured in the presence of fibronectin or RetroNectinTM.

46. The method according to claim 37, wherein the heterologous gene is a marker gene.

47. The method according to claim 37, wherein the heterologous gene is a therapeutic gene.